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General Unknown Screening by Ion Trap LC/MS/MS

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16. Abstract

During the investigation of aviation accidents, postmortem specimens from accident victims are submitted to the Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. The first, and perhaps most important, step in the analysis process is the initial screening of biological specimens for illicit, medically prescribed, and over-the-counter compounds that may be present and could have been the cause of the accident. Currently, our General Unknown Screening (GUS) procedure involves both gas chromatography/mass spectrometry (GC/MS), liquid chromatography/diode array detection (LC/DAD) and fluorescence detection techniques. Both techniques have inherent limitations that prevent the detection of certain types of compounds. LC/DAD, however, is more limited due to poor sensitivity and specificity. Therefore, our laboratory developed and validated an LC/MS/MS procedure that provides far superior sensitivity and specificity to that of LC/DAD. The combination of GC/MS with LC/MS/MS will allow for the detection of more compounds at lower concentrations than our current techniques.

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General Unknown Screening by Ion Trap LC/MS/MS

INTRODUCTION

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) is responsible under U.S. Department of Transportation Orders 8020.11B and 1100.2C to "conduct toxicological analysis on specimens from … aircraft accident fatalities" and "investigate … general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of … chemical [use]." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Forensic Toxicology Research Laboratory for toxicological analysis of various postmortem fluids and tissues.

Procedures utilized for the initial detection of unknown compounds in a biological sample, usually referred to as general unknown screening (GUS), employ a variety of analytical techniques.[1] GUS can involve various immunoassay methodologies, including radioimmunoassay (RIA), fluorescence polarization immunoassay (FPIA), and enzyme-linked immunosorbent assay (ELISA).[2] These immunoassay techniques are ideal for the detection of specific compounds in different drug classes like various drugs of abuse, or widely prescribed pharmaceuticals such as oxycodone; however, these techniques are limited to a specific and quite limited list of compounds. This limitation forces most laboratories to incorporate additional layers of analytical instrumentation into the GUS procedure so that a wider variety of compounds may be detected. These chromatographic techniques can be coupled to a variety of detectors such as mass spectrometers (MS), fluorescence, or ultraviolet (UV) diode array detectors (DAD). Gas chromatography-mass spectrometry (GC/MS) has been utilized in the GUS process for decades.[3-5] This technique is extremely useful due to the availability of large libraries containing hundreds of thousands of standardized spectra. However, GC/MS has limitations also. For example, large molecules that are thermally labile are not volatile enough to be amenable to this technique. As pharmaceutical compounds become larger and more complex this issue has become more prevalent. One viable solution to this problem is the addition of another analytical technique to the GUS procedure, liquid chromatography (LC). LC has been used for decades, as well and is specifically suited for the separation of large, thermally labile compounds.[5] The most common detector used in conjunction with LC is the DAD. Diode array detectors provide UV spectra for compounds that absorb UV radiation. These spectra are generally compound-dependant and can, therefore, be placed in a library and used for identifying unknown compounds in a specimen. However, DAD is not as specific as MS.[5] Numerous compounds may have spectra that look similar, making identification tedious, and this method of detection cannot identify compounds with no UV absorbance.

In recent years, the combination of LC with MS has become increasingly prevalent in many laboratories.[1,5-10] This combination provides an almost universal separation technique and the most sensitive and specific detector allowing for the detection of a wider variety of compounds than either GC/MS or LC/DAD alone. LC/MS will play a significant role in the future of GUS. Laboratories around the world have begun the process of phasing out LC/UV and replacing it with LC/MS methodology. Our laboratory created and validated an LC/MS/MS method and an associated library of compounds for use as a part of our GUS procedure, which is presented here.

MATERIALS AND METHODS

Reagents, Standards, and Supplies

All aqueous solutions were prepared using double deionized water (DDW), which was obtained using an ELGA, PURELAB Ultra water system (ELGA, Lowell, MA). All chemicals were purchased in the highest possible purity and used without any further purification. All solvents were of HPLC-grade and were obtained from Fisher Scientific (Fischer Scientific Co., Fair Lawn, NJ). Formic acid (97%) and ammonium formate were purchased from ICN (ICN Biomedicals, Inc., Irvine, CA) and Fisher Scientific, respectively. Analytical grade compound standards were obtained from a variety of sources as either 1 mg/mL liquid standards or pure powder standards. When analytical grade standards were not commercially available, drug standards were obtained via prescription. Pills were purchased, crushed, filtered, and diluted, resulting in solutions that were approximately 1 mg/mL for the compound of interest.

Instrumentation

Liquid Chromatographic/Mass Spectroscopic Conditions

Analyte separation was achieved using an Agilent 1200 series HPLC (Agilent Corp., Wilmington, DE) equipped

with a Security Guard C-8 guard column (4.0 x 3.0 mm i.d., 3 µm particles) from Phenomenex (Torrance, CA), followed immediately by a Hypersil Gold PFP (150 x 2.1 mm i.d., 5 µm particles) analytical column obtained from Thermofisher Scientific (Thermofisher Scientific Corp., San Jose, CA). Samples were injected using an Agilent G1367C autosampler. Identification and quantification were accomplished using a Thermofisher Scientific model LTQ XL electrospray ionization (ESI) linear ion trap mass spectrometer, which utilized nitrogen as the sheath and auxiliary gas.

For all determinations, the HPLC was operated in a gradient mode with a constant flow rate of 0.30 mL/min. The mobile phase employed consisted of acetonitrile with 0.10% formic acid (A) and 10 mM ammonium formate buffer with 0.10% formic acid (B). The gradient was set up as follows: initially, 5% A 95% B; at 5.0 min, 45% A 55% B; at 18 min 70% A 30% B; at 20 min 95% A 5% B; at 25 min 95% A 5% B; at 25.1 min 5% A 95% B; and at 30 min 5% A 95% B. The sample injection volume was held constant at 10 µL. The HPLC column was routinely allowed to equilibrate overnight prior to use. Following use, the column was washed with and stored in 50:50, acetonitrile:H_2O.

The operating conditions for the data collection segments of the MS were as follows: heated capillary temperature, 350°C; capillary voltage, 4.00 V; source current, 4.00 µA; sheath gas flow (nitrogen), 25; auxiliary gas flow (nitrogen) 5; spray voltage, 5.00 kV; multipole 00 offset, –3.25 V; lens 0 voltage, –5.50 V; multipole 0 offset, –5.25 V; lens 1 voltage, –8.00 V; gate lens voltage, –56.00 V; multiplier 1 offset, –11.50 V; multipole RF amplitude, 400 V; front lens, –5.75 V and 1 micro-scan having a maximum ion injection time of 100 msec. This segment was further split into 9 separate scan events. Scan event 1 collected full-scan data in the positive ion mode. Scan events 2-6 were data-dependant scans that collected MS/MS data following fragmentation of any ion from the positive-ion parent mass list that was encountered in scan event 1. Collision-induced dissociation (CID) of the precursor ions encountered from the positive-ion mass list using a collision energy of 35% produced MS/MS spectra that were compared to those in the library. Scan event 7 collected full-scan data in the negative ion mode. Scan events 8-9 were data-dependant scans that collected MS/MS data following fragmentation of any ion from the negative-ion parent mass list that was encountered in scan event 7.

Initially, precursor ions were identified for each compound investigated by infusing the analyte directly into the mobile phase, which was then introduced into the mass spectrometer at a flow rate of 0.30 mL/min. Following either $[M+H]^+$ or $[M-H]^-$ ion identification, the precursor ions were added to the appropriate mass list (positive or negative). Fragmentation at a collision energy of 35% provided an MS/MS spectra for each analyte of interest that was exported into the newly created library. Finally, retention times for each analyte were obtained through the injection of neat standards: one µL of a 10 µg/mL standard was injected onto the LC column under the conditions described above. This provided the retention time data that were then added to the MS method creating a retention time range in which the MS would target a particular compound.

Control of the HPLC system, integration of any chromatographic peaks, and communication with the mass spectrometer were accomplished via a personal computer using Xcalibur LC/MS software (Thermofisher Scientific Corp.). Unknown identification and report processing was accomplished using ToxID version 1.0 software (Thermofisher).

Sample Extraction

Controls were prepared and extracted in the following manner. Compounds were analyzed in groups of 10. Each group of compounds was prepared and analyzed at 4 different concentrations (1, 10, 100, and 1000 ng/mL), which provided an approximation of the limit of detection (LOD) for each compound. The 1000 ng/mL control concentration was prepared by first diluting 100 µL of each 1 mg/mL stock standard to 10 mL in a class A volumetric flask with DDW, providing an aqueous 10 µg/mL working solution. One mL of the 10 µg/mL working solution was diluted to 10 mL in a class-A volumetric flask with bovine whole blood, resulting in a 1000 ng/mL whole blood control. The 1000 ng/mL control was diluted by serial dilution using bovine whole blood as the diluent to create the remaining control specimens at concentrations of 100, 10, and 1 ng/mL.

Three mL aliquots of each control specimen were transferred to individual 15 mL screw-top vials. Six mL of 0.10 M phosphate buffer, pH 6.00, was added to each specimen. The mixture was then placed on a rotary mixing wheel and mixed for 15 min by simple rotation of the wheel at 15 rpm. Centrifugation at 2500 *g* for 30 min allowed for the removal of cellular debris and proteins. Following centrifugation, the extracts were transferred to Bond Elute Certify® solid-phase extraction (SPE) columns obtained from Varian (Varian Co., Harbor City, CA.) for the isolation of any basic compounds. The columns had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL 0.10 M phosphate buffer, pH 6.00. Care was taken not to dry the column prior to adding the extract. Column flow rates of 1-2 mL/min were maintained in

each SPE step using a Varian 24 port Cerex SPE processor (Varian Co., Harbor City, CA.) with a nitrogen pressure of 3 psi. Once each sample had passed through its respective column, the columns were washed with 1.00 mL of 1.00 M acetic acid and then dried completely with 25 psi nitrogen for 5 min. The columns were then washed with 6.00 mL of methanol. The methanol wash was collected in clean, labeled 10 x 100 mm culture tubes because it contains any acidic or neutral compounds that may have been present in the sample. Following collection of the methanol wash, the columns were again dried completely with 25 psi nitrogen for 5 min. The basic analytes were eluted off the Bond Elute Certify® columns with 3.00 mL of 2.0% ammonium hydroxide in ethyl acetate, which was prepared each day.

The previously collected methanol wash was evaporated to dryness in a TurboVap concentration workstation (Caliper Life Sciences, Hopkinton, MA) set at 40°C under a stream of dry nitrogen. Once dry, the contents of each tube were reconstituted with 3 mL of 0.10 M phosphate buffer, pH 6.00, vortexed, and transferred to Styre Screen SPE columns (United Chemical Technologies Inc., Bristol, PA.), which had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL 0.10 M phosphate buffer, pH 6.00. Care was taken not to dry the SPE column prior to extract addition. Column flow rates of 1 - 2 mL/min were maintained in each step using a Varian 24-port Cerex SPE processor with a nitrogen pressure of 1 psi. As each sample passed through its respective column, the columns were washed with 2 mL 0.10 M phosphate buffer, pH 6.00, and then dried completely with 25 psi nitrogen for 1 min. The columns were then washed by adding 1 mL of 1.0 M acetic acid and were then dried completely with 25 psi nitrogen for 2 min. The columns were then washed by adding 2 mL of hexane and were again dried completely with 25 psi nitrogen for 2 min. The acidic and neutral analytes were eluted from the Styre Screen columns with 3 mL of methylene chloride.

Eluents from both SPE extractions were evaporated to dryness in a TurboVap set at 40°C under a stream of dry nitrogen. Once dry, the contents of each tube were reconstituted in 50 µL of 50:50 acetonitrile:water. The two eluents from each sample were then transferred to separate LC/MS vials for analysis, resulting in an acid/neutral and a base vial from each specimen.

RESULTS AND DISCUSSION

This study established the LOD for 359 forensically valuable compounds in the newly created ion-trap LC/MS/MS library while also establishing the appropriate precursor and product ion for each compound and in which fraction (basic or acid/neutral) each compound should be expected following extraction. Acquisition of the mass spectra was achieved using a ThermoFisher Scientific LTQ XL linear ion-trap with an Ion Max™ ESI source in both positive and negative ionization modes within a single run. Full-scan data were collected throughout each run in both the positive and negative ionization modes, allowing for the possible identification of compounds not yet in the library. The newly-created library contains compound information including: product ion mass spectra, molecular weight, chemical structure, molecular formula, and CAS number. Limit of detection was defined by the lowest concentration that provided a signal-to-noise ratio of at least 3 and a search index match of at least 600, while simultaneously providing a reverse search index match of at least 700. The individual index values are calculated by the ToxID software and serve as an indicator of the quality of the match between the unknown mass spectrum and the mass spectrum contained within the library. This extensive amount of data has been tabulated and can be seen in Table 1.

There were interesting findings on both the mass spectrometry and the extraction side of this experiment. The linear ion trap is extremely sensitive and allowed for the identification of the majority of the compounds investigated at concentrations as low as 1 ng/mL. However, in a few instances this methodology proved to be unsatisfactory. For example, certain compounds such as tramadol ionize exceptionally well, but the chemical structure does not allow for fragmentation of the precursor ion in the linear ion-trap. The tramadol product ion and the precursor ion are identical, and the software can not differentiate between these two ions during analysis, preventing the identification of this compound when utilizing this methodology. Of the 369 compounds initially investigated, this phenomenon was encountered 6 times. The 6 compounds that could not be fragmented following precursor ionization included: fosinopril, meclofenamic acid, phenylbutazone, tenoxicam, terbutaline, and tramadol. The other mass spectrometry-related issue encountered during this work was compound ionization. Some compounds, under these specific mobile phase and mass spectrometry conditions, will not ionize or ionize poorly. Without a precursor ion there can be no product mass spectrum and therefore no compound identification. Of the 369 compounds initially investigated, this

phenomenon was encountered 4 times. The 4 compounds that could not be ionized under these conditions included: acamprosate, amiodarone, isosorbide, and simvastatin. The two limitations discussed above preclude the use of LC/MS alone as a comprehensive GUS technique. However, in combination with GC/MS, each of the problematic compounds mentioned can easily be detected, and the other 359 compounds in the library can be detected at lower concentrations than previously possible.

Ionization forming the precursor ion and subsequent fragmentation to form the product ion spectra allows for compound identification via library matching to a known spectrum. However, isolating the compounds contained within the library from a biological matrix and introducing these compounds into the mass spectrometer is a complex process. As can be seen in the extraction section (above), the procedure used was comprehensive, lengthy, and labor intensive. An effort to isolate compounds from a wide variety of chemical classes sometimes prevents the ability to detect specific drugs at low levels. This is the nature of GUS. Additionally, some compounds were detected in both the basic and the acidic fractions following extraction. Clonazepam, for example, was found to have an LOD of 10 ng/mL in the basic fraction. However, only 60% of the total number of area counts for this compound was seen in the basic fraction. Forty percent of the dose was seen in the acidic fraction. If 100% of the dose was contained within either fraction, the LOD obtained would be lower. This phenomenon was observed with 4 of the compounds investigated including: clonazepam, tetrahydrocannabinol, irbesartan, and ramipril. A possible solution to this problem would be to eliminate, or significantly simplify, the extraction procedure. Preliminary work has begun to evaluate a simple "crash-and-shoot" extraction process. In this procedure, acetonitrile is added to a biological specimen to precipitate any proteins present. The sample is centrifuged, and a small portion of the acetonitrile is injected into the LC/MS/MS system. This procedure has shown significant improvements in extraction efficiency for the limited number of compounds investigated.

The newly-validated GUS library developed with LC/MS/MS technology has been in use on a trial basis for the previous 12 months. Over this time period, prior to injection on our currently used LC/UV system, a small portion of each extract was removed and injected onto the LC/MS/MS system so that a direct comparison of the results would be possible. It became immediately evident that the new analytical methodology would be much more sensitive than the currently used technique. Numerous compounds that would not have been detected by the current LC/UV and GC/MS combination have been confirmed after screening positive by LC/MS/MS. This encouraging result provides more evidence in favor of a fundamental shift in the analytical techniques used for the purpose of GUS.

CONCLUSIONS

A compound library constructed with spectra obtained from an ion-trap LC/MS/MS for 359 forensically important compounds has been created. This methodology has the potential to replace our current LC technique and allow for the detection of compounds at lower concentrations than previously possible. The LOD for each compound in the library has been established, as well as the fraction in which the compound will be seen following extraction. The future addition of other compounds is a relatively simple procedure as standards for new pharmaceutical compounds are obtained. An additional benefit is the collection of full-scan MS data throughout the run. These data provide the molecular weight of any compound encountered that is not currently in the library, which could be useful for unknown identification. When combined with our current GC/MS procedure, the newly-validated LC/MS screening technique will allow for the detection of more compounds at lower concentrations than is currently possible.

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Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
11-hydroxy-delta-9-THC	13.30	331.2	295.3	100	Acid
11-nor-9-carboxy-delta-9-THC	14.70	345.2	299.2	100	Acid
2-Hydroxyethylflurazepam	9.59	333.1	305.1	10	Base
4-Hydroxynordiazepam	8.79	287.1	259.1	1	Base
6-Acetylcodeine	9.80	342.2	225.1	1	Base
6-Acetylmorphine	8.05	328.1	211.2	10	Base
6-B-Naltrexol	7.47	344.2	308.2	1	Base
7-Amino-Clonazepam	7.70	286.1	250.2	1	Base
7-Amino-Flunitrazepam	8.41	284.1	256.2	10	Base
Acebutolol	7.70	337.3	260.2	10	Base
Acetaminophen	6.25	152.1	110	100	Base
Albuterol	6.10	240.1	148.1	100	Base
Alfuzosin	8.71	390.3	235.1	1	Base
alpha-Hydroxymidazolam	10.60	342.1	203	1	Base
Alprazolam	10.20	309.1	274.2	1	Base
Alprenolol	11.73	250.2	116.2	1	Base
Aminorex	6.70	163	120	1	Base
Amitriptyline	15.30	278.2	233.2	1	Base
Amlodipine	12.10	409.2	320.1	1000	Base
Amoxapine	12.10	314	271.2	10	Base
Amphetamine	7.72	136.2	91.2	10	Base
Anhydroecgonine	4.00	168.1	137.1	> 1000	
Anhydroecgonine Methyl Ester	5.01	182.1	151.1	10	Base
Apomorphine	4.60	268.2	237.2	1	Base
Aripirazole	14.85	448.2	285.1	1	Base
Astemizole	12.30	459.3	218.2	1	Acid
Atenolol	6.45	267.1	190.2	10	Base
Atomoxetine	12.70	256.1	147.9	10	Base
Atorvastatin	12.2	559.3	440.3	10	Acid
Atropine	8.60	290.2	124.2	1	Base
Azacyclonol	11.20	268.2	132.1	10	Base
BDB	7.90	194	135	1	Base
Benazepril	12.43	425.2	351.1	100	Base
Benzocaine	9.27	166.1	138.2	100	Acid
Benzoylecgonine	7.54	290.2	168.1	100	Base
Benzphetamine	13.74	240.2	91	1	Base
Benzthiazide	10.51	430	308	10	Acid
Betaxolol	6.60	308.3	116.2	1	Base
Bisoprolol	10.20	326.3	116.2	1	Base
Bromazepam	8.97	316	288.1	10	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Brompheniramine	11.70	321.1	276.1	1	Base
Buprenorphine	13.40	468.4	414.3	1	Base
Bupropion	11.40	240.1	184	1	Base
Bupropion Metabolite	11.04	242.1	186	1	Base
Buspirone	11.13	386.3	122.1	1	Base
Butabarbital	8.47	211.1	168.1	100	Acid
Butorphanol	9.90	328.2	282.2	1	Base
Candesartan	15.95	441.3	263.1	1000	Base
Cannabinol	19.10	311.2	223.1	1	Base
Carbamazepine	9.40	237.1	194.1	1	Acid
Carbinoxamine	10.60	291.1	202	1	Base
Carisoprodol	10.10	261.1	176.2	1	Acid
Carvedilol	14.38	407.2	283.1	1	Base
Cathinone	6.49	150	105	10	Base
Celecoxib	14.30	382.3	303.1	100	Acid
Cetirizine	18.03	403.2	201	1	Base
Chlorcyclizine	14.82	301	201	1	Base
Chlordiazepoxide	9.80	300.1	227.1	1	Base
Chloroquine	13.60	320.3	247.2	100	Base
Chlorophacinon	13.12	373.1	201.1	1000	Acid
Chlorpheniramine	11.80	275.1	230.1	1	Base
Chlorpromazine	16.20	319.1	239.2	100	Base
Chlorprothixene	16.44	316.2	271.1	1	Base
Chlorzoxazone	9.26	168	168	1000	Acid
Cimetidine	6.12	253.2	159.1	1	Base
Cinnarizine	20.95	369.2	167.1	1	Base
cis-4-Methylaminorex	8.36	177	134	1	Base
Cisapride	13.90	466.2	184.1	1	Base
Citalopram	13.80	325.2	262.2	1	Base
Clenbuterol	9.52	277.1	203.1	1	Base
Clobazam	8.25	301.1	259.2	10	Acid
Clomipramine	17.05	315.2	270.2	10	Base
Clonazepam	10.40	316.1	270.1	10	Acid
Clonidine	7.38	230.1	230.1	1000	Base
Clopidogrel	14.24	322.1	212	1	Base
Clorazepate	9.98	271.1	243.1	10	Base
Clozapine	12.29	327.1	270.1	10	Base
Cocaethylene	12.20	318.1	196.1	1	Base
Cocaine	11.00	304.2	182.1	1	Base
Codeine	7.42	300.1	215.1	1	Base
Coumetetrayl	12.50	293.2	175.2	10	Acid
Cyclobenzaprine	14.90	276.2	231.2	10	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
d8-Amphetamine	7.72	144.1	97	1	Base
d8-Methamphetamine	8.27	158.1	124	1	Base
Delta-9-THC	19.20	315.1	259.3	100	Acid
Demoxepam	8.98	287.2	180	10	Acid
Desalkylflurazepam	10.30	289.1	261.1	1	Base
Desipramine	14.00	267.2	236.2	10	Base
Desmethylcitalopram	12.47	311.2	262.1	1	Base
Desmethylmetoprolol	7.39	254.1	177	1	Base
Dextromethorphan	12.00	272.2	215.2	1	Base
Diazepam	11.30	285.3	257.2	1	Base
Dihydrocodeine	7.20	302.1	201.2	1	Base
Dihydroergotamine	7.10	584.3	270.3	10	Base
Diltiazem	12.90	415.1	178.1	1	Base
Di-N-desmethylcitalopram	11.98	297.1	262.1	1	Base
Diphenhydramine	13.40	256.1	167.1	1	Base
Dipyrone	6.14	310.1	191	> 1000	
Disopyramide	10.10	340.3	239.2	1	Base
Donepezil	12.20	380.2	362.2	1000	Acid
Dothiepin	14.10	296.2	225	1	Base
Doxazosin	12.80	452.3	344.2	1	Base
Doxepin	13.50	280.1	235.1	10	Base
Doxylamine	10.40	271.1	182.1	1	Base
Duloxetine	10.25	298	267	100	Base
Ecgonine-Methyl-Ester	1.94	200.1	182	10	Base
EDDP	16.11	278.2	249.2	1	Base
EMDP	14.80	264.2	235.1	1	Base
Enalapril	9.70	377.2	234.1	> 1000	
Ephedrine	7.10	166.1	135	100	Base
Eserine	7.83	276.1	219.1	1	Base
Estazolam	9.84	295.1	267	1	Base
Ethotoin	8.27	205.1	106.1	100	Acid
Etomidate	11.20	245.2	141.1	1	Base
Ezetimide	12.23	410.3	201.1	> 1000	
Famotidine	5.72	338.1	259.1	10	Base
Felbamate	7.95	239.1	178.1	100	Acid
Felodipine	9.87	384.1	356	10	Acid
Fendiline	17.81	316.2	212.2	1	Base
Fenfluramine	12.60	232.1	159	1	Base
Fenoprofen	12.55	241.1	197.1	1	Acid
Fentanyl	13.40	337.3	188.2	1	Base
Fexofenadine	14.25	502.3	466.4	100	Base
Flecainide	14.13	415.1	301.1	1	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Fluconazole	7.86	307.1	238	5	Base
Flunitrazepam	10.80	314.1	268.2	1	Base
Fluoxetine	16.80	310.1	148	10	Base
Fluphenazine	16.54	438.3	171.2	1	Base
Flurazepam	12.40	388.2	315.2	1	Base
Fluvastatin	8.18	412.3	266.1	100	Acid
Fluvoxamine	11.90	319.1	226.1	10	Base
Furazolidine	8.39	243.1	112.9	100	Acid
Furosemide	9.70	329.2	285	1	Base
Gabapentin	6.58	172.1	137	> 1000	
Gemfibrozil	14.34	249	121.1	100	Acid
Glimepiride	8.30	491	352.2	100	Acid
Glipizide	7.99	444.2	319	10	Acid
Glyburide	8.40	492	367	1000	Acid
Guafenasin	7.68	216.1	163.1	100	Acid
Halazepam	13.35	353.1	325	1	Base
Haloperidol	14.60	376.1	165	1	Base
Heroin	9.90	370.2	268.2	1	Base
Hydrochlorothiazide	7.50	295.9	268.9	1000	Acid
Hydrocodone	8.30	300.1	199.2	1	Base
Hydroflumethazide	8.46	330	303	1	Base
Hydromorphone	6.70	286.1	185.1	10	Base
Hydroxy-Alprazolam	9.27	325.3	279.1	1	Base
Hydroxychloroquine	10.31	336.2	247	1	Acid
Hydroxy-Triazolam	9.51	359.1	313.1	1	Base
Hydroxyzine	7.90	375.2	201.1	1	Base
Ibogaine	12.40	311.2	174.1	1	Base
Imipramine	14.80	281.1	86.1	1	Base
Indomethacin	8.91	358.1	174.2	100	Acid
Irbesartan	12.10	429.3	207	10	Acid
Isoniazid	1.98	138.1	138.1	1000	Base
Isotretinoin	8.73	301.2	255.2	1000	Acid
Ketamine	8.80	238.1	207.1	1	Base
Ketoprofen	8.02	255.2	209.2	100	Acid
Ketorolac	8.70	256.1	105.1	10	Acid
Labetalol	11.20	329.2	207.2	1	Base
Lamotrigine	8.93	256	211	1000	Base
Lansoprazole	10.31	298.2	266.1	10	Base
Levorphanol	8.64	258.2	201.1	10	Base
Lidocaine	9.10	235.2	86.1	1	Base
Lometazepam	9.50	335.1	289.1	1	Acid
Loratadine	14.08	383.2	337.2	1	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Lorazepam	9.90	321.2	275.1	1	Acid
Losartan	10.50	423.3	207.1	100	Acid
Loxapine	12.71	328.1	271.1	1	Base
LSD	10.43	324.2	223.1	1	Base
Malathion	8.60	347.7	284.8	100	Acid
Maprotiline	14.66	278.2	250.2	1	Base
MBDB	9.04	208.2	177	1	Base
MDA	8.30	180	135	10	Base
MDEA	9.30	208.2	163	1	Base
MDMA	8.60	194.1	163.1	1	Base
Meclizine	23.51	391.2	201.1	1	Base
Medazepam	13.30	271.2	242	1	Base
Mefloquine	17.80	379.2	321.2	1	Base
Meperidine	10.60	248.1	220.2	10	Base
Mephobarbital	9.54	245.1	181	10	Base
Mepivocaine	8.41	247.1	98	1	Base
Meprobamate	8.30	219.1	158.1	1	Acid
Mescaline	7.50	212.1	180.2	10	Base
Mesoridazine	11.40	387.2	126.2	10	Base
Metaproterenol	1.30	212.2	152.2	1	Base
Metaxalone	9.99	222.1	161.1	100	Acid
Methadone	16.37	310.1	265.1	1	Base
Methamphetamine	8.30	150.1	119.2	1	Base
Methaqualone	10.22	251.1	132	10	Base
Methcathinone	6.30	164	133.1	1	Base
Methoxyverapamil	15.68	485.4	333.3	1	Base
Methylephedrine	7.01	180.1	135.1	1	Base
Methylphenidate	9.84	234.1	84.2	1	Base
Methysergide	9.44	354.2	237.1	1	Base
Metoclopramide	9.00	300.2	227.1	1	Base
Metolazone	9.57	366.1	259	10	Acid
Metoprolol	8.82	268.1	191.1	1	Base
Mexiletine	9.76	180.1	58.2	1	Base
Mianserin	13.02	265.2	208.1	1	Base
Miconazole	13.30	417.2	159.1	10	Base
Midazolam	12.10	326.3	291.2	1	Base
Minoxidil	8.80	210.2	137.1	100	Base
Mirtazapine	9.94	266.1	195.1	1	Base
Modafinil	8.96	167.2	167	100	Acid
Molsidomine	6.26	243.1	86.2	1	Base
Montelukast	17.91	586.2	422.2	1000	Base
Morphine	5.50	286.1	201.2	100	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
N-Acetylprocainamide	6.73	278.2	205.2	1	Base
Nadolol	7.69	310.2	254.1	1	Base
Nalbuphine	7.88	358.3	272	1	Base
Nalorphine	7.09	312.2	270.2	1	Base
Naloxone	6.20	328.2	310.2	100	Base
Naltrexone	7.51	342.2	270.2	1	Base
Naphazoline	10.07	211.2	211.1	1000	Base
N-Desmethyl-cis-tramadol	9.64	250.1	189.1	10	Base
N-Desmethylclomipramine	15.43	301.2	270.1	10	Base
N-Desmethylflunitrazepam	10.19	298.1	278	10	Base
N-Desmethyltrimipramine	14.70	281.2	86	1	Base
Nebivolol	16.70	406.3	151.2	100	Base
Nefazodone	16.58	471.1	274.1	1	Base
N-Ethylamphetamine	8.73	164.1	119	1	Base
Nicotine	2.81	163.1	132.1	1	Base
Nifedipine	10.00	329.1	270.1	100	Acid
Nimodipine	13.03	417.1	294.1	10	Acid
Nisoldipine	13.74	389.1	357	100	Acid
Nitrazepam	9.73	282.2	236.2	10	Base
Nitrendipine	12.71	359.1	236	10	Acid
Nitrofurantoin	7.85	237	152	1000	Acid
Nizatidine	5.88	332.1	232.2	10	Base
Norbenzoylecgonine	7.65	276.1	154.1	100	Base
Norbuprenorphine	10.33	414.3	340.2	10	Base
Norchlordiazepoxide	12.56	271.1	229.1	1000	Acid
Norclomipramine	15.88	301.2	270.2	1	Base
Norcocaethylene	11.64	304.2	182.1	10	Base
Norcocaine	10.62	290.1	168	1	Base
Norcodeine	7.07	286.2	225.1	1	Base
Nordiazepam	10.40	271.1	243	10	Base
Nordoxepin	12.96	266.1	235.1	1	Base
Norfentanyl	8.63	233.1	83.9	1	Base
Norfluoxetine	16.00	296.1	134.1	100	Base
Norketamine	8.33	224.2	179	1	Base
NOR-LSD	10.50	310.2	209.1	1	Base
Normeperidine	9.87	234.1	160.1	1	Base
Normorphine	3.85	272.2	211.1	1000	Base
Noroxycodone	7.78	302.1	229.1	10	Base
Noroxymorphone	4.72	288.1	215.2	100	Base
Norpropoxyphene	18.20	308.2	100	100	Base
Norsertraline	15.20	293.9	276.9	100	Base
Norsildenafil	10.69	461.3	311.1	1	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Nortriptyline	14.50	264.1	233.1	1	Base
Norvenlafaxine	8.51	264.1	201.1	1	Base
Norverapamil	15.21	441.3	165	1	Base
Olanzapine	10.38	313.2	256	1	Base
Olmesartan	11.6	559.1	429.1	1000	Base
Omeprazole	9.17	346	198.1	1	Base
Ondansetron	11.10	294.2	170.2	1	Base
Opipramol	12.62	364.3	171.2	1	Base
Orlistat	23.82	496.2	319.2	1000	Acid
Orphenadrine	13.85	270.1	181.1	1	Base
Oxazepam	9.70	287.1	241	10	Base
Oxazolam	9.77	329.2	271.1	10	Base
Oxybutynin	14.81	358.3	124.1	1	Base
Oxcarbazepine	9.12	253.2	208.1	10	Acid
Oxprenolol	10.02	266.2	225.1	1	Base
Oxycodone	8.00	316.1	256.2	1	Base
Oxymetazoline	13.20	261.3	205.1	100	Base
Oxymorphone	5.86	302.1	284.2	10	Base
Paliperidone	10.61	427.3	207.1	1	Base
Pantoprazole	9.52	384.1	200	10	Base
Paroxetine	14.27	330.1	192.1	1	Base
PCP	12.73	244.1	86.1	1	Base
Penbutolol	15.69	292.2	236.1	1	Base
Pentazocine	11.01	286.3	218.2	1	Base
Perphenazine	15.41	404.2	171.1	10	Base
Phendimetrazine	8.21	192.1	192.1	10	Base
Pheniramine	9.47	241.2	196.1	1	Base
Phenmetrazine	7.86	178.1	178.1	1	Base
Phenoxybenzamine	16.61	304.2	120	10	Base
Phentermine	8.42	150.1	91	10	Base
Phenylephrine	3.19	168.1	150	1000	Base
Phenylpropanolamine	6.00	152.1	117.1	1000	Base
Phenyltoloxamine	13.70	256.2	133.2	10	Base
Pindolol	4.60	249.2	116.2	10	Base
Pioglitazone	11.10	357.2	134.1	1	Base
Piroxicam	12.53	330	266.2	100	Base
PMA	8.20	166.1	121	1	Base
PMMA	8.68	180.1	149.1	1	Base
Prazepam	11.70	325.2	271.1	1	Base
Prazosin	12.03	384.2	247.2	1000	Base
Primidone	7.36	219.1	162	100	Base
Procainamide	6.17	236.1	163.1	1	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Promazine	14.40	285.2	86.1	10	Base
Promethazine	14.98	285.3	240.3	1	Base
Propafenone	14.27	342.2	116.2	1	Base
Propoxyphene	16.25	340	266.2	10	Base
Propranolol	11.90	260.2	183	1	Base
Protriptyline	13.22	264.1	233.1	1	Base
Pseudoephedrine	7.05	166.1	148.1	1000	Base
Pyrilamine	7.10	286.2	241.2	1	Base
Quazepam	15.38	387.1	354.1	1	Base
Quetiapine	12.03	384.2	253.2	1	Base
Quinapril	13.81	439.3	234.1	100	Base
Quinidine	11.04	325.2	253.1	10	Base
Quinine	10.72	325.2	253.3	1	Base
Rabeprazole	10.31	298.1	266.1	100	Base
Ramelteon	10.04	260.2	204.1	1	Acid
Ramipril	12.00	417.2	234.1	100	Base
Ranitidine	6.84	315.1	176.1	10	Base
Risperidone	11.26	411.2	191.1	1	Base
Rosiglitazone	10.50	358.2	135.1	10	Base
Rosuvastatin	10.52	480.1	418.1	10	Acid
Salmeterol	13.17	416.3	380.3	1	Base
Scopolamine	8.00	304.2	138.1	1	Base
Selegiline	9.37	188.2	119.1	1000	Base
SERTIS	15.30	316.1	285.1	1	Base
Sertraline	17.30	306.2	275.1	1	Base
Sibutramine	18.11	280.2	139	1	Base
Sildenafil	10.88	475.2	311.1	10	Base
Sotalol	3.10	273.1	213.1	10	Base
Stanozolol	11.10	329.3	121	10	Base
Strychnine	8.21	335.2	264.1	1	Base
Sulpiride	7.09	342.2	112	1	Base
Sumatripan	7.83	296.1	251.1	10	Base
Tacrine	8.82	199.2	199.1	1	Base
Tadalafil	10.36	390.1	268	10	Acid
Tamsulosin	11.84	409.2	271.1	10	Base
Telmisartan	15.57	515.3	276.2	1	Base
Temazepam	10.40	301.1	255	1	Base
Terazosin	8.60	388.2	290.1	1	Base
Terfenadine	12.30	472.4	436.4	1	Base
Thiopental	10.48	241.1	101	1000	Acid
Thioridazine	19.60	371.2	126.1	1	Base
Thioridazine	23.51	391.2	201.1	1	Base

Table 1. Analytical data for each of the 359 compounds in the LC/MS/MS library (Continued)

Compound name	RT	Precursor ion	Product ion	LOD*	Fraction**
Thiothixene	15.29	444.3	335.2	1	Base
Timolol	9.30	317.1	261.1	1	Base
Tizanidine	6.84	254.1	254.1	10	Base
Tolterodine	15.50	326.3	284.1	10	Base
Topiramate	9.25	357	281.8	100	Acid
Torsemide	9.54	349.1	264.1	10	Base
Trazodone	11.26	372.2	176.1	1	Base
Triamterene	8.70	254.2	194.8	1000	Base
Triazolam	10.20	343.1	308.2	1	Base
Trichloromethiazide	9.43	379.9	306	10	Acid
Trifluprom azine	18.11	353.2	308.1	1	Base
Trimethobenzamide	9.47	389.2	166.1	1	Base
Trimethoprim	8.11	291.2	230.2	1	Base
Trimipramine	16.13	295.2	100.1	1	Base
Valsartan	11.11	436.3	306.1	100	Acid
Vardenafil	11.19	489.2	299.1	10	Base
Varenicline	6.62	212.2	169.1	100	Base
Venlafaxine	10.49	278.3	215.2	1	Base
Verapamil	15.80	455.1	303.2	1	Base
Warfarin	11.63	309.1	163	10	Acid
Zaleplon	9.75	306.2	264	10	Acid
Zimelidine	10.74	317	272	1	Base
Ziprasidone	11.44	413.1	194	1	Base
Zolpidem	10.70	308.2	263.2	1	Base
Zopiclone	8.83	389	345.2	1	Base

* All concentrations are presented with the units of ng/mL

** Denotes the fraction from which the compound was recovered during the extraction process